



TITLE:

HIV-1 viral infectivity factor interacts with TP53 to induce G2 cell cycle arrest and positively regulate viral replication

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CITATION:

Izumi, Taisuke ...[et al]. HIV-1 viral infectivity factor interacts with TP53 to induce G2 cell cycle arrest and positively regulate viral replication. Proceedings of the National Academy of Sciences 2010, 107(48): 20798-20803

ISSUE DATE:

2010-11-11

URL:

<http://hdl.handle.net/2433/131335>

RIGHT:

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Fig. S1 Vif directly binds to TP53 *in vivo*. HEK293T cells were co-transfected with pNL-A1 and expression vectors for HA-TP53 or HA-TP53(Gln22,Ser23), which is defective in MDM2 binding. Cell lysates were immunoprecipitated with anti-HA mAb followed by immunoblotting with the indicated Abs (upper panels). Total cell lysates were also subjected to immunoblotting with the indicated Abs (lower panels).

Fig. S2 Definition of the Vif domain that interacts with TP53. HEK293T cells were co-transfected with expression vectors for Vif and Vif deletion mutants together with pcDNA3/HA-TP53. Cell lysates were immunoprecipitated with anti-HA mAb followed by immunoblotting with the indicated Abs (upper panels). Total cell lysates were also subjected to immunoblotting with the indicated Abs (lower panels). Arrowhead indicates TP53, whereas an asterisk indicates heavy chain. We could not detect co-precipitation of the Δ 23-74 mutant with TP53 due to low expression of this mutant (lane 4).

Fig. S3 GST pull-down assays demonstrate interaction of Vif with TP53 *in vitro*. *In vitro* translated HA-TP53, HA-TP53(Gln22,Ser23), and HA-A3G were precipitated with GST, GST-Vif, or GST-Vif Δ 22 beads followed by immunoblotting

with the indicated Abs (upper panels) GST or GST-Vif proteins were also stained with Coomassie Blue (lower panel).

Fig. S4 Vif blocks the binding of MDM2 to TP53. HCT116 *p53*^{-/-} cells were co-transfected with expression vectors for Vif and HA-TP53 as indicated. Cell lysates were immunoprecipitated with anti-HA mAb followed by immunoblotting with indicated Abs (upper panels). Total cell lysates were also subjected to immunoblotting with the indicated Abs (lower panels).

Fig. S5 The mutants of NL4-3 Vif to HXB2 defective for the ability to induce G2 arrest lost the ability to overcome the MDM2 activity, whereas the mutant of HXB2 to NL4-3 capable of inducing G2 arrest recuperated the ability to overcome the MDM2 activity. Luciferase reporter assays were performed as shown in Fig. 1 and 3.

Fig. S6 HXB2 Vif can also bind to TP53 *in vivo*. HEK293T cells were co-transfected with pDon-Vif and expression vectors for HA-TP53. Cell lysates were immunoprecipitated with anti-HA mAb followed by immunoblotting with the indicated Abs (upper panels). Total cell lysates were also

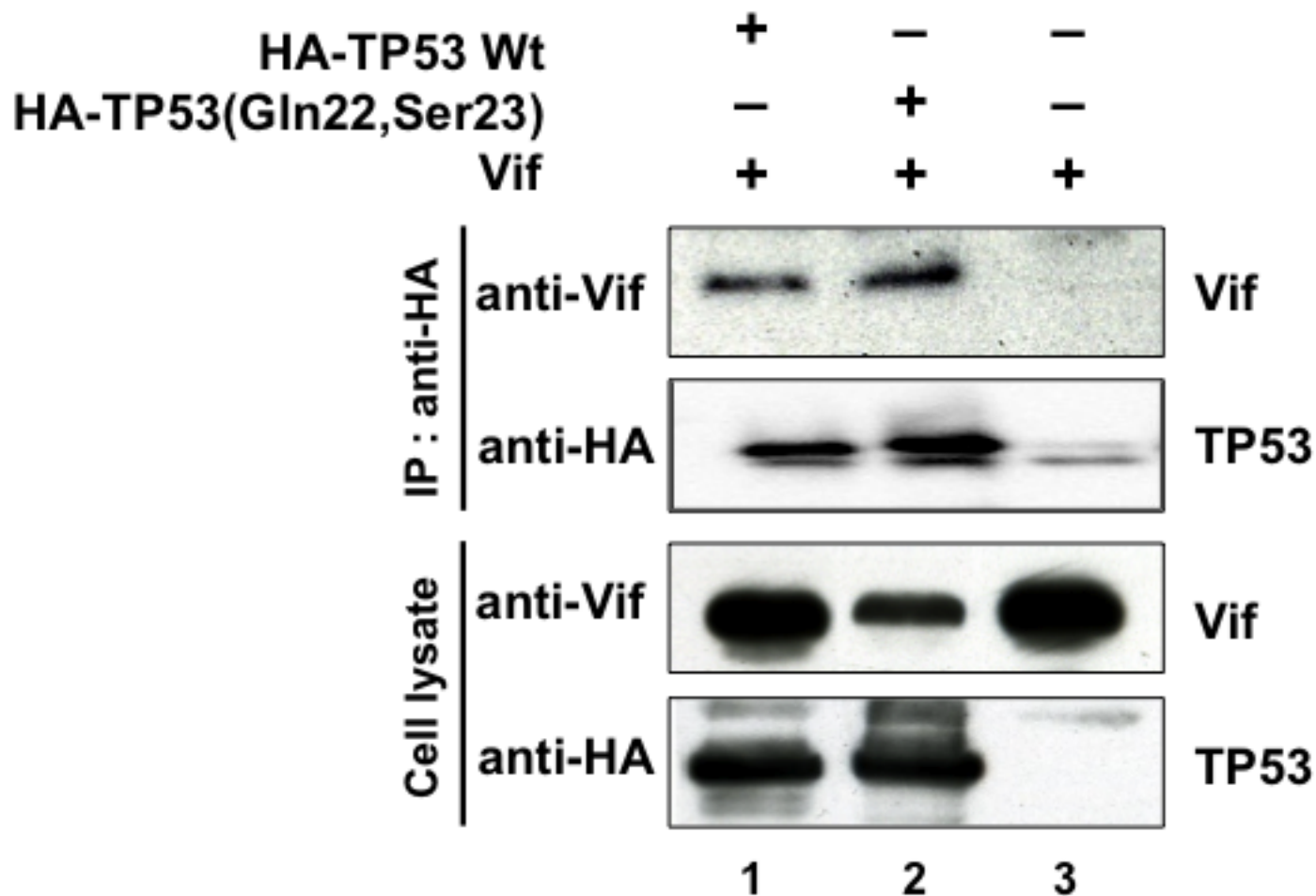
subjected to immunoblotting with the indicated Abs (lower panels).

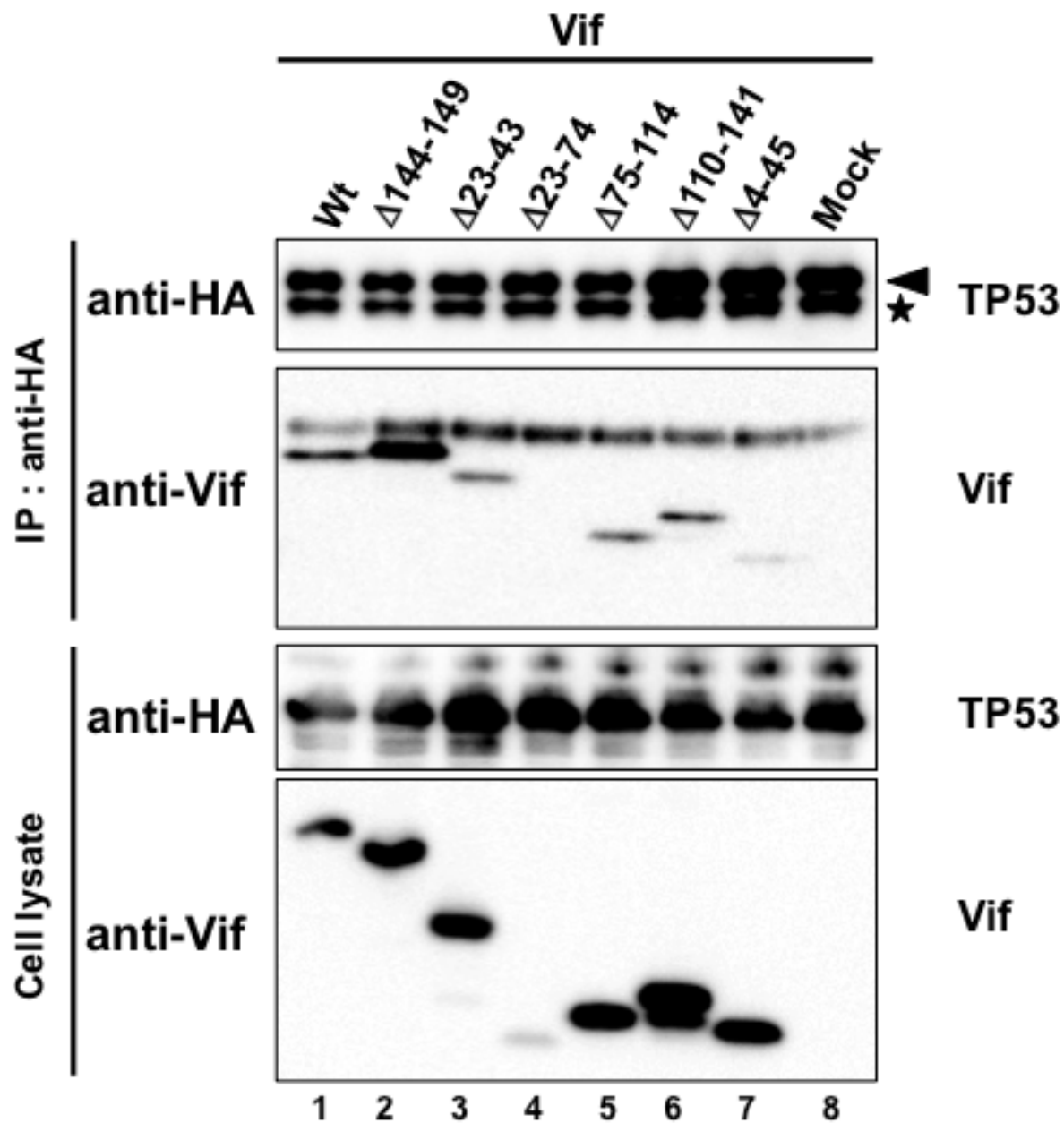
Fig. S7 Replication kinetics of HIV-1 in Jurkat T cell line. Jurkat cells were challenged with normalized stocks of wild-type and a chimeric Vif-expressing NL4-3, which express Nef protein, and viral replication was monitored as the supernatant accumulation of p24 as shown in Fig. 4.

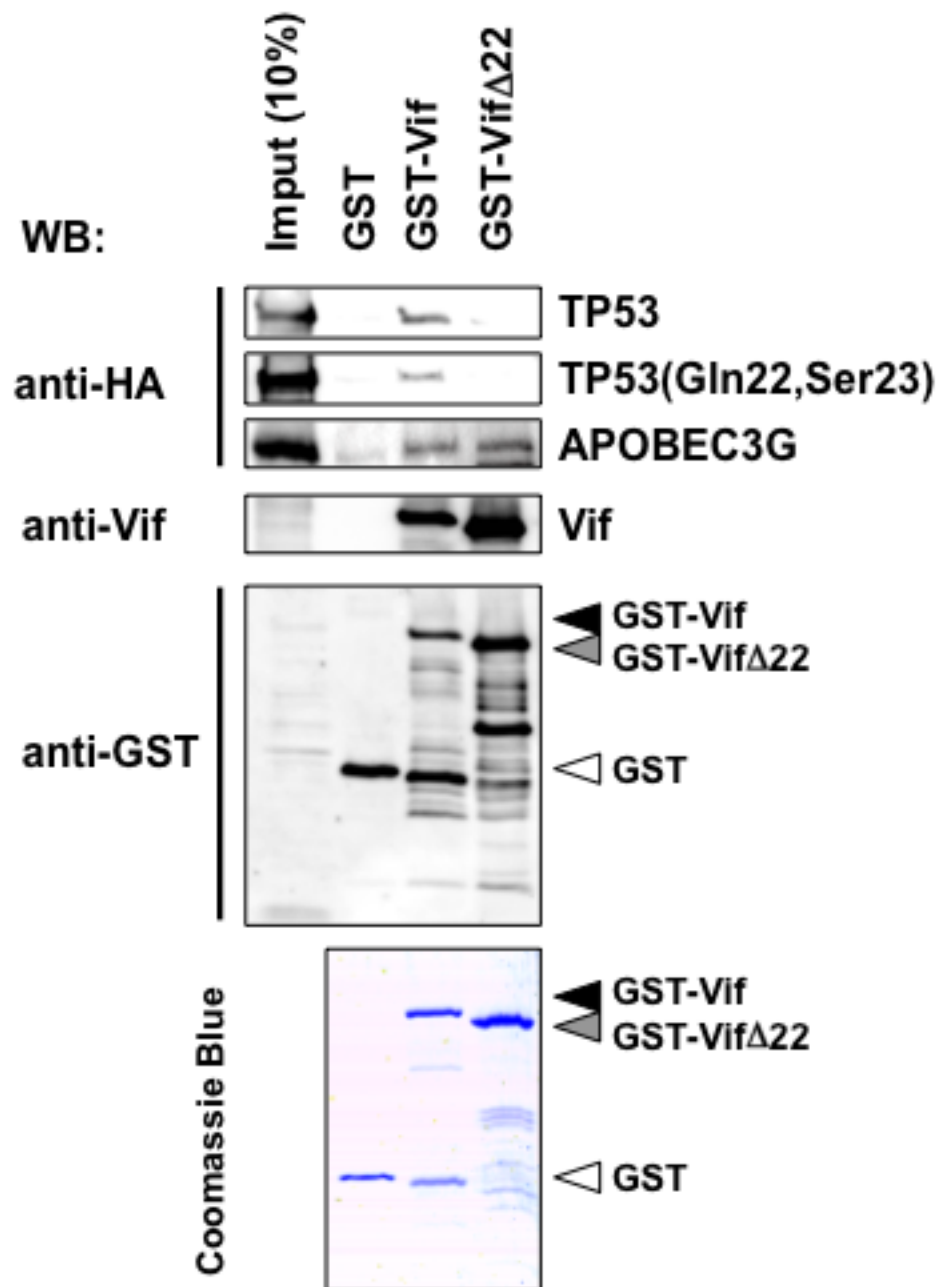
Fig. S8 Schematic summary depicting the functional interaction between Vif and the TP53/MDM2 pathway. Stabilization and activation of TP53 by Vif leads to a negative feedback loop (indicated by red lines) as well as G2 arrest (indicated by blue lines).

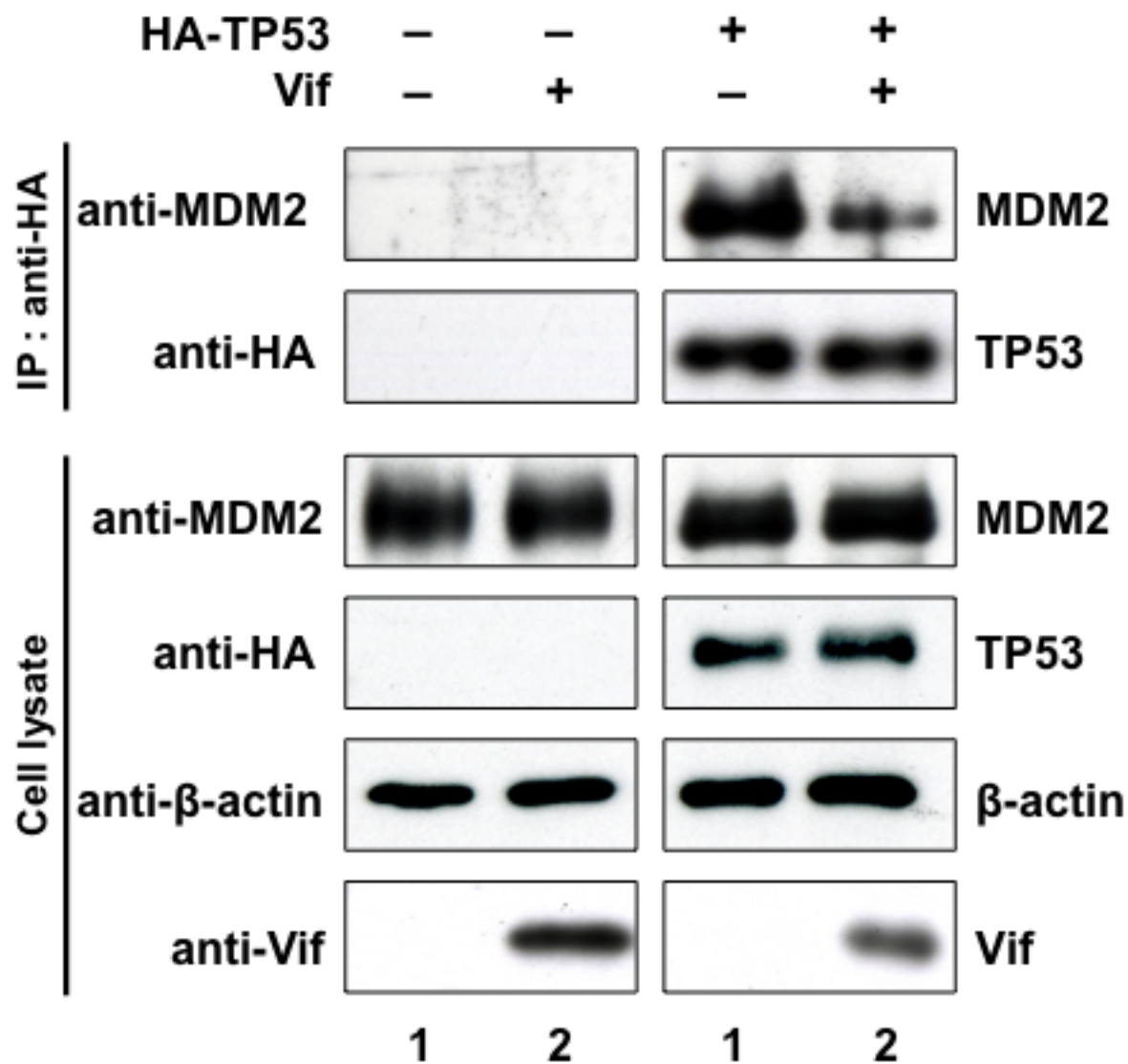
Fig. S9 Chimeric Vif reduced cellular levels of A3G to the same extent as NL4-3 Vif. HEK293T cells were cotransfected with expression vectors for A3G and HIV-1. Cell lysates were subjected to immunoblotting with the indicated Abs.

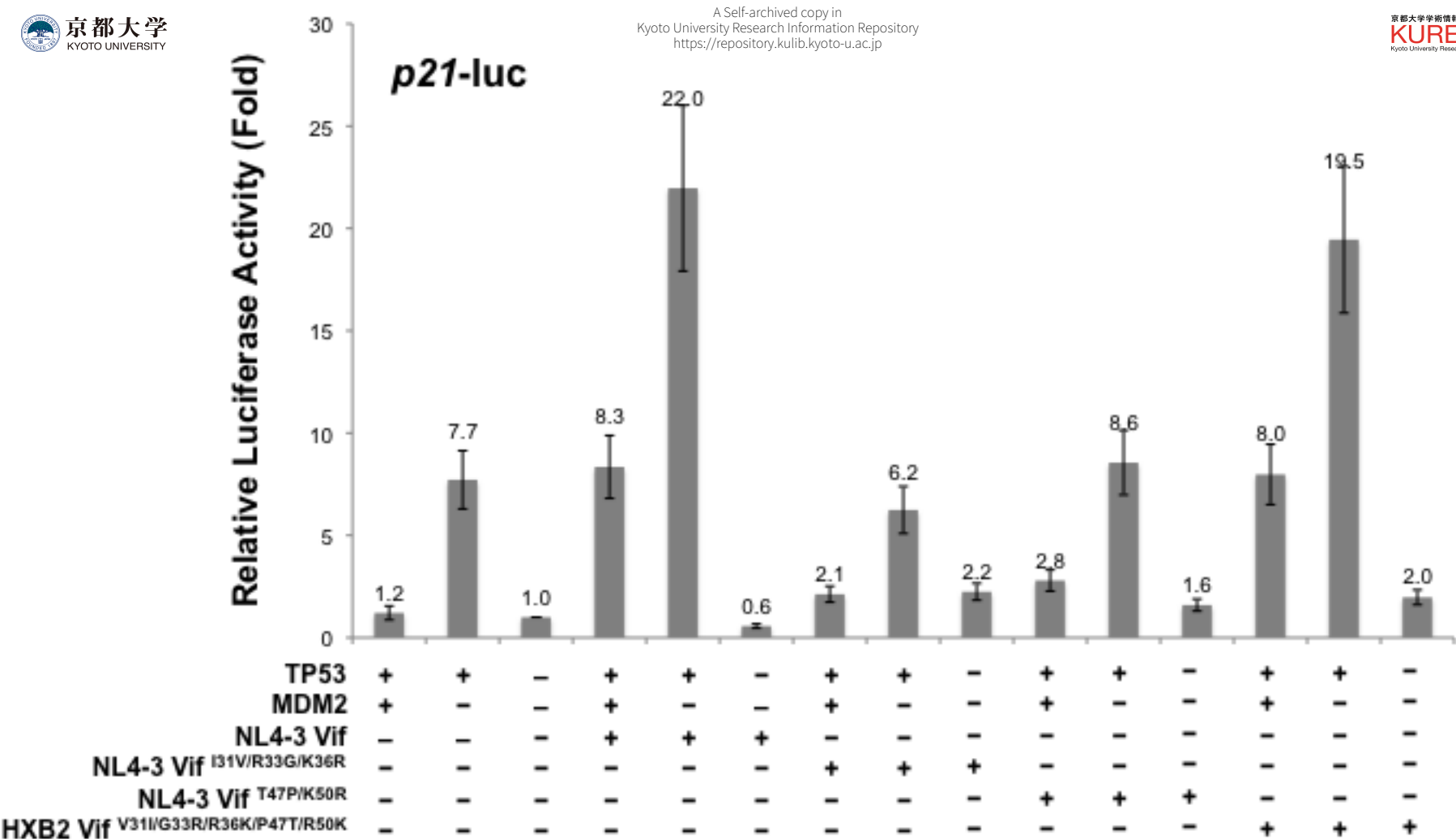
Fig. S10 GFP-Vif reduced cellular levels of A3G. HEK293T cells were cotransfected with expression vectors for A3G and GFP-fused NL4-3 or HXB2 Vif. Cell lysates were subjected to immunoblotting with the indicated Abs.











HA-TP53	—	+	—	+	+	+	+	+
NL4-3 Vif	—	—	+	+	—	—	—	—
HXB2 Vif	—	—	—	—	+	—	—	—
NL4-3 Vif I31V/R33G/K36R	—	—	—	—	—	+	—	—
NL4-3 Vif T47P/K50R	—	—	—	—	—	—	+	—
NL4-3 Vif Δ 22	—	—	—	—	—	—	—	+

